Effect In-Ovo Injection (MethioGrow) Liquid Methionine in Broiler Breeder Eggs On Hatching Characters and Growth Performance

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Abstract

Amino acids could be use due to its positive effects on several physiological and biochemical parameters, which key adapting function of metabolic processes during early development. Methionine is typically the first-limiting amino acid for protein requirement in poultry diets. Investigators are shown that Methionine concentrations in the blood plasma, and tissue of newly hatched control chicks were lower than those of chicks that received amino acid mix in egg The present study was conducted to study the effect of injecting methionine on the chicks growth performance, and immunoglobulin (IgG and IgA). Fertile 375 eggs of (Ross-308) broiler breeder strain were obtained at 30 weeks of age from Kosar Company, Taq Taq/Erbil. All eggs were weighted, incubated, and distributed randomly into five treatments. Our results indicate the egg injection with MethioGrow have significantly effect the body weight, feed conversion ratio, hatchability and Immunoglobulin's (IgG, IgM) at 7 and 17 days old. But feed intake was not significant affected by adding the MethioGrow.

Key words: Methionine, Ovo injection, hatchability.

تأثير حقن بيض امهات الدجاج اللاحم بالمثيونين السائل (MethioGrow) على خصائص التفريخ وأداء النمو.

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الخلاصة

يمكن استخداماالاحماض الامينية نظرا لتاثيرها الايجابي على العديد من المعلمات الفيسيولوجية و الكيموحيوية، و التي تعمل على تكيف وظيفة عمليات التمثيل الغذائي أثناء التطور الجنيني المبكر. المثيونيين هو عادة اول حامض اميني محدد لمتطلبات البروتين في اعلاف الدواجن. أظهر باحثون ان تركيز المثيونيين في بلازما الدم و انسجة الافراخ حديثة الفقس كانت اقل من تلك الموجودة في الافراخ التي تلقت مزيج الاحماض الامينية في البيض. اجريت هذه الدراسة لمعرفة تاثير حقن المثيونيين على اداء نمو الافراخ ، و الغلوبيولين المناعي (**IgG, IgA**). تم الحصول على 375 بيضة مخصبة من سلالة أمهات التسمين (**ROSS-308**) في عمر 30 أسبوع من شركة كوسار للدواجن الواقعه في منطقة طقطق/اربيل. تم وزن كل البيض و حضنة و توزيعة عشوائيا الى خمسة معاملات. تشير النتائج الى ان حقن البيض باستخدام مادة (**MethioGrow**) لة تاثير كبير على وزن الجسم، نسبة التحويل الغذائي، الفقس، و الغلوبيولين المناعي (**IgG, IgA**) في عمر 7 و 17 يوم. بينما تناول لم يتاثر باضافة(**MethioGrow**).

Introduction

Chick growth can be increased with in Ovo injection of amino acids (Isa, 2018). Amino acids (AA) could be utilized due to its positive effects on several physiological and biochemical parameters, key adapting function of metabolic processes during early development (Leandro et al, 2010). Several factors affect avian embryogenesis, such as in Ovo nutrients injection (Shafey et al, 2014), methionine (Met) is typically the first-limiting AA for protein requirement in poultry diets. Methionine is considered to play main roles in the body (Kidd, 2004). Many types of research have assessed different treatments during the pre- and immediate post-hatch periods with the intention to improve the growth rate and body weight. The essential amino acid is used in important metabolic processes such as protein synthesis. Methionine is the third most limiting amino acid, especially in a low crude protein diet (Ebrahimi et al., 2017).

Ohta et al. (1999) showed that Methionine concentrations in the blood plasma and tissue of newly hatched control chicks were lower than those of chicks that received amino acid mix in-Ovo. It was reported that *in-Ovo* injections of Methionine increasing hatching weight (Bhanja and Mandal, 2005), and increasing final weight in broiler chicks (Bhanja et al., 2012).

Studies demonstrated that methionine constructively affects the immune system, improving both cellular and humoral immune response (Swain & Johri, 2000). It was reported that methionine requirements for optimal immunity are higher than for optimal growth (Shini & Bryden, 2005). One of the mechanisms proposed to explain methionine interference in the immune system is the proliferation of T cells (Kinscherf, et al., 1994).

The present investigation was conducted to study methods of injecting methionine and the effect of in-Ovo injection on growth performance, immunoglobulin IgG and IgA.

Materials and Methods

The experiment protocol was approved by the University of Sulaimani / College of Agricultural Engineering Science/Animal science. Fertile 375 eggs of (Ross-308) broiler breeder strain were obtained at 30 weeks of age from Kosar Company, Taq Taq/Erbil. All eggs were collected from the same breeder flock and weighed on a balance with 0.1 g precision and eggs with a weight of 60 ± 1 g were incubated at 37.8 °C and 63 percentage RH. at 1 d of incubation. Fertile eggs were distributed randomly into five different treatment groups with three replication each including 15 eggs. Treatment groups were negative control (no injection), positive control (distilled water), on the 7th day of incubation, the eggs were candled, and the infertile ones or those containing only dead embryos were removed. The in Ovo injection solutions were control without injection, injected with 0.5 ml deionized water, injected with 15 mg MethioGrow in 0.5 ml deionized water, injected with 20 mg MethioGrow in 0.5 ml deionized water in two time at 7 and 17 days. After injection, holes were sealed with adhesive tape and incubation continued. After injection, eggs were allocated to different treatment groups. Chicks were weighed after hatching by using a balance with 0.1 g precision all groups were fed *ad libitum*. Composition and chemical analysis of experimental diets in starter, grower and finisher periods are presented in (Table 1).

The chicks are reared using three different levels of diets as follows:

Starter diet: during age period 7-11 days including 23% crude protein and 2900 Kcal/kg metabolizable energy. Grower diet: during age period 12-25 days including 21.5% crude protein and 3000 Kcal/kg metabolizable energy. Finisher diet: during age period 26-56 days including 20% crude protein and 3175 Kcal/kg metabolizable energy.

Data collection:

Live body weight:

The birds were weighed per replicate at the start of the experiment and subsequently on daily basis (7, 14, 21, 28, 35, and 42 days of age).

Body weight gain:

At the beginning of the experiment, all of chicks were weighed. At the end of each week, every replicate's chicks were weighed, and deduced from their first week's weight. Therefore, weekly weight gain can be measured. The same method was applied to measure weight gain in the periods overall period (7-42) days old.

Feed intake:

To determine feed intake, specific amounts of ration were weighed and given to the birds for every replicate at the beginning of week. At the end of week, the rest of the ration from every replicate were weighed and deducted from the original ration, so weekly-consumed diet can be yielded. Feed intake at 14, 21, 28, 35 and entire feed intake during 14-42 days old was calculated as well as the mortality of each replication was calculated.

Feed conversion ratio:

After measuring feed intake and body weight gain over a week, the following equitation used to determine feed conversion ratio:

$$\frac{Feed\ intake\ over\ a\ week}{week's\ beginning\ weight - \ week's\ ending\ weight}$$

Feed conversion ratio analysis was calculated in overall period (1-42) days old, and also the whole period intervals

Maglumi IgM（CLIA） Kit Use purified IgM and IgA antigen to label ABEI, and use anti-IgM monoclonal antibody to label FITC. Sample, Calibrator, Control, ABEI Label, FITC Label and magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated at 37℃, forming complexes; after sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time. Subsequently, the starter reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of IgM present in controls or samples.

All data will statistically analyzing according to the Completely Randomized Design (CRD) by the (Xlstate, 2019) system and the differences between the means of groups will separating by Duncan Multiple Range Test (Duncan, 1955) statements of statistical significance are basing on ($P \leq 0.05$).

Result

In Ovo injection of MethioGrow have significant effect on body weight at 7 and 17 days old (P<0.05) (Table2). Relative chick weight at 7 days was changed by injections MethioGrow and showed higher weight compered to another injection. Results in (Table2) show injection in 7 days have significant effect on body weight until final weight (marketing weight), Al Daraji et al. (2012), Bhanja et al. (2004), and Keralapurath et al. (2010) reported that in Ovo injection amino acid included lysine, and that methionine injection did not affect growth performance of birds. On the other hand, Foye et al. (2006), Kadam et al. (2008), Gaafar et al. (2013), and Shafey et al. (2014) reported that in Ovo injection amino acid injection increased the growth performance of broilers. (Table 3) There are not significant different between groups in feed intake, Feed conversion ratio was decrees by MethioGrow treatments day 7 and positive control compared to another treatments (P<0.05).

Hatchability rate increased more in the control and 17 day MethioGrow injection than in the negative control and MethioGrow groups (P<0.05) (Table 4)

The results of immunohistochemically analysis Table (5) shows in Ovo injection of MethioGrow on the expression of IgG and IgM. These data indicated that MethioGrow 17 day and MethioGrow 7 day injected under the air sac significantly increased ($p < 0.05$) with other injection treatments. However, when the IgM gene expressed higher MethioGrow 7 day than other group the differences compared with other injected groups were significant between MethioGrow 17 day and MethioGrow with other injection.

Discussion

There are few studies on optimum time and site for injection of AA into egg. Adequate time and position for AA injection into egg was evaluated in Experiment 2. The results obtained suggest that the AA solution injected into egg at day 7 may be an effective means to increase chick size without decreasing hatchability. Also, better results were obtained when the AA were injected into the egg yolk on Day 7 of incubation. The earlier studies showed that nutrient injection volume, injection time, and injection depth might affect the growth performance parameters of broilers. In the current study, in Ovo injection carried out on the 7th day and 17 days of incubation into the air sac of eggs did not decrease hatchability. At the end of this study, performance parameters were not changed since there might not have been the presence of any stress factors during the experimental period for chicks. The results of this study showed that in Ovo injection of MethioGrow, had no negative effects on performance parameters. In our studies, secretory IgA antibodies producing cells were also detected in chickens

Research, IgM content decreased in the met deficiency group, which suggests that dietary met deficiency could lead to the impairment of primary immune responses. Moreover, IgG, secreted by B cells, is the main antibody isotype found in blood and extracellular fluids, enabling it to control body tissue infections (Hirano *et al*., 1986). IgG contributes directly to immune responses including the neutralization of toxins and viruses (Lindner *et al*., 2012). Functionally, IgG is generated mainly in secondary antibody responses (Shimizu *et al*., 1992).

Different results about performance parameters have been obtained from different previous in Ovo injection studies. For example, Al Daraji et al. (2012), Bhanja et al. (2004), and Keralapurath et al. (2010) reported that in Ovo amino acid included lysine, and that MethioGrow injection did not affect growth performance of birds. On the other hand, Foye et al. (2006), Kadam et al. (2008), Gaafar et al. (2013), and Shafey et al. (2014) reported that in Ovo amino acid injection increased the growth performance of broilers.

Acknowledgments

The authors acknowledge the financial assistance provided by Kosar Company for execution of this investigation **Table 1: Composition and chemical analysis of experimental diets in starter, grower and finisher periods**

Ingredients	Starter	Grower	Finisher
Wheat flour	15.90	16.00	15.50
Soybean	34.00	30.00	25.50
Yellow corn	43.35	46.60	51.88
Miavit oremix	2.50	2.40	2.30
Choline	0.20	0.20	0.18
Enzyme	0.05	0.00	0.00
Anticoccidea	0.00	0.05	0.05
Antitoxin	0.10	0.05	0.10
Oil	2.00	3.00	3.00
Limestone	1.80	1.70	1.50
D.C.P.	0.10	0.00	0.00
total	100	100	100
Chemical analysis of diets			
Metabolizable energy Kcal/kg	2900	3000	3175
Crude Protein	23 %	21.0 %	20 %
Crude fat	5.6 %	5.3 %	5.3 %
Crude fiber	2.9 %	2.7%	2.6%
Moisture	11.7 %	11.8%	11.8%
Crude Ash	5.7 %	5.9%	5.8%
Phosphorus	1%	5.55%	0.55%
Lysine	0.61 %	1.29 %	1.16 %
Methionine	0.50 %	0.51 %	0.4 %
Meth+ Cyst.	1.33 %	0.99 %	0.77 %

Table 2: Effects of in Ovo injection on MethioGrow on live bodyweight

Traits Treatment	B.w.d.1	B.w.w.1	B.w.w.2	B.w.w.3	B.w.w.4	B.w.w.5	B.w.w.6
Negative control	49.233 ± 0.669 c	140.667 ± 1.202 c	180.667 ± 1.202 c	350.00 ± 2.887 d	462.000 ± 2.646 e	1241.667 ± 22.048 c	2541.667 ± 22.048 c
Positive control 7 day	48.700 ± 0.173c	155.000 ± 1.764 b	195.000 ± 1.764 b	353.000 ± 1.453 d	567.667 ± 1.856 c	1430.000 ± 22.048 b	2800.000 ± 9.866 b
MethioGrow 7 day	52.367 ± 0.669a	169.667 ± 0.333 a	209.667 ± 0.333 a	413.33 ± 3.756 b	697.667 ± 4.333 a	1620.333 ± 1.453 a	2950.00 ± 28.868 a
Positive control 17 day	50.733 ± 0.145 b	149.933 ± 2.067 b	189.933 ± 2.067 b	395.333 ± 2.906 c	551.333 ± 3.756 d	1209.333 ± 5.812 c	2563.33 ± 18.559 c
MethioGrow 17 day	51.267 ± 0.393 ab	152.667 ± 2.887 b	192.667 ± 2.887 b	432.33 ± 1.732 a	593.667 ± 1.453 b	1391.667 ± 20.817 b	2582.00 ± 28.868 c

Negative control = no injection; positive control = injection of 0.2 mL distilled water; MethioGrow = 100 µl metheogrow; SEM - standard error of mean; A+-b - Means for the same treatment and effect or with different letter differ significantly respectively (P<0.05); B.W= body weight, w= Week.

Table3: Effects of in Ovo injection of MethioGrow on feed intake, and feed conversion ratio of broiler chicks

Treatment / Traits	Negative control	MethioGrow 7 day	MethioGrow 17 day	Positive control 7 day	Positive control 17 day
Feed intake (g)	4519.72 ± 51.41 a	4436.0 ± 60.90 a	4477.91 ± 39.77 a	4758.33 ± 318.3 a	4573.77 ± 62.22 a
Feed conversion ratio (g/g)	1.857 ± 0.148 a	1.231 ± 0.033 c	1.542 ± 0.097 b	1.873 ± 0.061 a	1.330 ± 0.072 bc

Negative control = no injection; positive control = injection of 0.2 mL distilled water; MethioGrow = 100 µl MethioGrow; SEM - standard error of mean.; a-b - Means for the same treatment and effect or with different letter differ significantly respectively (P<0.05).

Table 4: Effects of in Ovo injection of MethioGrow on Hatchability, and Mortality of broiler chicks

Treatment / Traits	Negative control	MethioGrow 7 day	MethioGrow 17 day	Positive control 7 day	Positive control 17 day
Hatchability	0.840 ±0.012 ab	0.828 ±0.006 ab	0.835 ±0.000 ab	0.823±0.000 a	0.845±0.000 b
Mortality	2.41±0.116 c	2.43±0.237 c	2.64±0.122 c	2.09±0.075 c	2.96±0.072 c

Negative control = no injection; positive control = injection of 0.2 mL distilled water; MethioGrow = 100 µl metheogrow; SEM - standard error of mean; a-b - Means for the same treatment and effect or with different letter differ significantly respectively (P<0.05).; Negative control = no injection; positive control = injection of 0.2 mL distilled water; MethioGrow = 100 µl metheogrow; SEM - standard error of mean.

Table 5: Effects of in Ovo injection on MethioGrow on IgG, and IgM of broiler chicks

Treatments / Traits	Negative control	MethioGrow 7 day	MethioGrow 17 day	Positive control 7 day	Positive control 17 day
IgG	0.600 ± 0.058 ab	0.760 ± 0.345 ab	1.100 ± 0.115 a	0.433 ± 0.088 b	0.42 ± 0.079 b
IgM	5.333 ± 0.88 c	20.000 ±1.15 a	13.333 ± 0.88 b	4.733± 0.994 c	4.87 ± 0.19 c

Negative control = no injection; positive control = injection of 0.2 mL distilled water; c = 100 µl MethioGrow; SEM - standard error of mean.; a-b - Means for the same treatment and effect or with different letter differ significantly respectively (P<0.05).

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